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| 1. REPORT DATE (DD-MM-YYYY)<br>25-05-2000  |             | 2. REPORT DATE<br>FINAL |                               | 3. DATES COVERED (From - To)<br>MAR 1999 - FEB 2000    |   |
| 4. TITLE AND SUBTITLE<br>Children's Hospital of Pittsburgh<br>Histocompatibility Center<br>Microfabricated CE Chips to Make More Cost-Effective HLA Class I and Class II Molecular Typing  |             |                         |                               | 5a. CONTRACT NUMBER                                    |   |
|  |             |                         |                               | 5b. GRANT NUMBER<br>N00014-99-1-0616                   |   |
|  |             |                         |                               | 5c. PROGRAM ELEMENT NUMBER                             |   |
|  |             |                         |                               | 5d. PROJECT NUMBER                                     |   |
| 6. AUTHOR(S)<br>Massimo Trucco, M.D.   |             |                         |                               | 5e. TASK NUMBER  |   |
|  |             |                         |                               | 5f. WORK UNIT NUMBER                                   |   |
|  |             |                         |                               |  |   |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)<br>Children's Hospital of Pittsburgh<br>Rangos Research Center<br>3460 Fifth Avenue<br>Pittsburgh, PA 15213-3205  |             |                         |                               | 8. PERFORMING ORGANIZATION<br>REPORT NUMBER<br><br>341 |   |
| 9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)<br>Department of the Navy<br>Office of Naval Research<br>800 North Quincy Street<br>Arlington, VA 22217-5660   |             |                         |                               | 10. SPONSOR/MONITOR'S ACRONYM(S)                       |   |
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| 12. DISTRIBUTION AVAILABILITY STATEMENT<br><br>Approved for Public Release; Distribution is Unlimited.   |             |                         |                               |  |   |
| 13. SUPPLEMENTARY NOTES  |             |                         |                               |  |   |
| 14. ABSTRACT<br>The activities of the Children's Hospital of Pittsburgh Histocompatibility Center can be classified under three major categories: 1) the repository service; 2) the molecular typing service; and 3) the research activities aimed at improving the technical support on which the previous two services are working. This year in the Repository, where blood samples from volunteers as bone marrow donors are collected from more than 125 recruiting centers nationwide, 161,019 samples were processed. We stored 483,057 vials in three sets of freezers (one sample per donor each freezer), while 247,991 were sent out to other Typing Laboratories for molecular HLA typings. In our laboratories, 10,723 typings were performed with a very high Q.C. standard, as monitored by National Marrow Donor Program. Our Center's research branch synthesized all the oligonucleotides to be used as PCR primers or labelled probes to perform molecular HLA typing. DNA sequencing was also performed when hybridization results were not optimal. Alternatives for storing blood samples (e.g., blood spots on filter paper) or for enhancing typing capabilities (e.g., microchip technology to expedite PCR procedures) were successfully implemented or improved to the point of justifying their future implementation. |             |                         |                               |  |   |
| 15. SUBJECT TERMS<br><br>Histocompatibility, bone marrow, transplantation, PCR, microchips   |             |                         |                               |  |   |
| 16. SECURITY CLASSIFICATION OF:  |             |                         | 17. LIMITATION OF<br>ABSTRACT | 18. NUMBER<br>OF PAGES                                 | 19a. NAME OF RESPONSIBLE PERSON                           |
| a. REPORT  | b. ABSTRACT | c. THIS PAGE            | UU                            |  | Massimo Trucco, M.D.                                      |
| UU   | UU          | UU                      |                               |  | 19b. TELEPHONE NUMBER (Include area code)<br>412-692-6570 |

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# CHILDREN'S HOSPITAL OF PITTSBURGH HISTOCOMPATIBILITY CENTER

AWARD NUMBER: N00014-99-1-0616

Total Samples Received, Stored and Shipped from the Repository from 03/22/99 to 02/29/00

| SAMPLES                   | NUMBER<br>(March 1999) | NUMBER<br>(April 1999) | NUMBER<br>(May 1999)    |
|---------------------------|------------------------|------------------------|-------------------------|
| Stored                    | 19,903                 | 18,510                 | 13,184                  |
| Shipped (DR - Class II)   | 10,628                 | 9,555                  | 10,584                  |
| Shipped (AB - Class I)    | 10,494                 | 9,235                  | 10,522                  |
| Shipped (Q.C. - Serology) | 130                    | 129                    | 102                     |
| Destroyed                 | 2,432                  | 649                    | 569                     |
|                           |                        |                        |                         |
| SAMPLES                   | NUMBER<br>(June 1999)  | NUMBER<br>(July 1999)  | NUMBER<br>(August 1999) |
| Stored                    | 14,427                 | 10,339                 | 8,660                   |
| Shipped (DR - Class II)   | 11,456                 | 13,334                 | 14,902                  |
| Shipped (AB - Class I)    | 8,550                  | 7,200                  | 9,155                   |
| Shipped (Q.C. - Serology) | 127                    | 0                      | 0                       |
| Destroyed                 | 922                    | 2,219                  | 1,954                   |
|                           |                        |                        |                         |

| <b>SAMPLES</b>            | <b>NUMBER</b><br><b>(September 1999)</b> | <b>Number</b><br><b>(October 1999)</b> | <b>Number</b><br><b>(November 1999)</b> |
|---------------------------|--|--|---|
| Stored                    | 9,839                                    | 13,367                                 | 14,212                                  |
| Shipped (DR - Class II)   | 14,624                                   | 12,631                                 | 16,713                                  |
| Shipped (AB - Class I)    | 8,153                                    | 9,300                                  | 11,560                                  |
| Shipped (Q.C. - Serology) | 0  | 0                                      | 0                                       |
| Destroyed                 | 913                                      | 3,725                                  | 0                                       |
|                           |  |  |   |
| <b>SAMPLES</b>            | <b>NUMBER</b><br><b>(December 1999)</b>  |  |   |
| Stored                    | 13,841                                   |  |   |
| Shipped (DR - Class II)   | 17,344                                   |  |   |
| Shipped (AB - Class I)    | 12,391                                   |  |   |
| Shipped (Q.C. - Serology) | 0  |  |   |
| Destroyed                 | 0  |  |   |
|                           |  |  |   |
| <b>SAMPLES</b>            |  | <b>Number</b><br><b>(January 2000)</b> | <b>Number</b><br><b>(February 2000)</b> |
| Stored                    |  | 12,889                                 | 11,848                                  |
| Shipped (AB/DR + QC)      |  | 8510 + 262 = 8,772                     | 10,104 + 296 = 10,400                   |
| Destroyed                 |  | 0                                      | 0                                       |

**LEGEND:**

- Stored samples (Multiply number stored x 3 because of triplicate aliquots)
- Shipped samples (Separated by AB and DR samples until 12/99)
- Shipped samples (Combined AB and DR samples, plus Q.C. samples, beginning January 2000)
- Shipped samples (Q.C. - Serology for NMDP Special Study 11/98 - 6/99)
- Destroyed because no longer eligible, so that blood sample must be removed from NMDP list.

# CHILDREN'S HOSPITAL OF PITTSBURGH HISTOCOMPATIBILITY CENTER

AWARD NUMBER: N00014-99-1-0616

Total Samples Typed and Results Sent from 03/22/99 to 02/29/00

|             | Total | Priority 1 | Priority 2 | Priority 3 | No Makes | Navy |
|-------------|-------|------------|------------|------------|----------|------|
| <b>1999</b> |       |            |            |            |          |      |
| March       | 1,105 | 70         | 1,034      | 0          | 1        | 0    |
| April       | 608   | 227        | 375        | 6          | 0        | 0    |
| May         | 1,021 | 123        | 894        | 3          | 1        | 0    |
| June        | 878   | 175        | 699        | 4          | 0        | 0    |
| July        | 962   | 76         | 886        | 0          | 0        | 0    |
| August      | 819   | 90         | 714        | 12         | 3        | 0    |
| September   | 817   | 68         | 746        | 3          | 0        | 0    |
| October     | 820   | 48         | 772        | 0          | 0        | 0    |
| November    | 820   | 78         | 742        | 0          | 0        | 0    |
| December    | 1229  | 30         | 1199       | 0          | 0        | 0    |
|             |       |            |            |            |          |      |
| <b>2000</b> |       |            |            |            |          |      |
| January     | 820   | 31         | 789        | 0          | 0        | 0    |
| February    | 824   | 29         | 789        | 6          | 0        | 0    |

# CHILDREN'S HOSPITAL OF PITTSBURGH HISTOCOMPATIBILITY CENTER

AWARD NUMBER: N00014-99-1-0616

Research Completed from 03/22/99 to 02/29/00

| Month/<br>Year: | Oligonucleotides<br>Synthesized: | ABI/PE<br>Sequencing: | Taqman Probes: | Miscellaneous Research Projects:  |
|-----------------|----------------------------------|-----------------------|----------------|---|
| Mar '99         | 64                               | 97                    | 0              | <p>Evaluation and implementation of Amersham-Pharmacia Biotech Microspin G-25 columns for purification of oligonucleotides following synthesis and deprotection.</p> <p>Performed quarterly Quality Control on all automated equipment.</p>   |
| Apr '99         | 77                               | 184                   | 2              | <p>Replaced HPLC column and updated instrument to optimally perform fluorescent-labelled oligonucleotide purification. Performed quality control on updated instrument.</p>   |
| May '99         | 53                               | 238                   | 4              | <p>Purchased and installed one new G3 Macintosh computer required for updated software for DNA sequencers.</p> <p>Installed new data collection and sequencing software.</p> <p>Began troubleshooting communication difficulties between new sequencing collection software and the instrument.</p> |
| Jun '99         | 72                               | 205                   | 2              | <p>Performed quarterly Quality Control on all automated equipment.</p>  |
| July '99        | 64                               | 408                   | 0              | <p>Continued to trouble-shoot and evaluate sequencing hardware and software.</p> <p>Purchased and installed a second G3 Macintosh from ABI to compare internal hardware components.</p>   |

| Month/<br>Year: | Oligonucleotides<br>Synthesized: | ABI/PE<br>Sequencing: | Taqman Probes: | Miscellaneous Research Projects:  |
|-----------------|----------------------------------|-----------------------|----------------|---|
| Aug '99         | 52                               | 144                   | 8              | Installation, optimization and evaluation of ABI HLA typing kit incorporating sequence-based typing strategies. Typing performed on 15 patient blood samples and results compared to SSOP typing results for confirmation.  |
| Sep '99         | 127                              | 129                   | 0              | Resolved communication conflicts with new Macintosh computer and DNA sequencer. Both new computers and instruments functioning optimally using updated software. Passed quality control check with known samples.   |
| Oct '99         | 54                               | 183                   | 0              | Performed quarterly Quality Control and annual preventive maintenance on all automated equipment.<br><br>Installed new ABI Genescan software for use on the DNA sequencers.<br><br>Began and completed trouble-shooting of data collection software for fragment analysis on DNA sequencers.    |
| Nov '99         | 39                               | 111                   | 0              | New matrices run and installed on sequencer 2.  |
| Dec '99         | 41                               | 234                   | 0              | Resolved software difficulties on sequencer 2 and installed new matrix.<br><br>Performed quarterly Quality Control and annual preventive maintenance on all automated equipment.  |
| Jan '00         | 79                               | 319                   | 0              | Installed new matrix on sequencer 2.  |
| Feb '00         | 50                               | 370                   | 0              |   |
| Mar '00         | 74                               | 317                   | 0              | Installed new matrix on sequencer 1.<br><br>Performed quarterly quality control and annual preventive maintenance on all automated equipment.<br><br>Completed contracted service and issued inventory of all TaqMan probes to Neogen.<br><br>Attended BioCad perfusion chromatograph training. |

| Month/<br>Year: | Oligonucleotides<br>Synthesized: | ABI/PE<br>Sequencing: | Taqman Probes: | Miscellaneous Research Projects:   |
|-----------------|----------------------------------|-----------------------|----------------|--|
| Apr '00         | 66                               | 254                   | 0              | Began preliminary investigation into separation of recombinant AAV from cell culture using perfusion chromatography/ |